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Olfactory Axon Pathfinding: Who Is the Pied Piper?

Mammalian olfactory sensory neurons that express a particular odorant receptor (OR) project axons to the same few glomeruli in the olfactory bulb. In this issue of *Neuron*, Vassalli et al. (2002) use OR minigenes that coexpress histochemical markers and show that the determinants in the sensory neurons required to generate the stereotyped olfactory bulb map are the same as those needed for appropriate expression of the OR.

In mammals, odorants are sensed by ORs located on olfactory sensory neurons in the olfactory epithelium of the nasal cavity. The sensory neurons send signals to the olfactory bulb that are then relayed to the olfactory cortex and other brain areas. Studies employing OR genes have provided numerous insights into the mechanisms underlying olfactory perception (reviewed in Buck, 2000). There are ~1000 genes that encode ORs, but each neuron expresses only one OR gene, and different alleles of that gene are expressed in different neurons. The olfactory epithelium is divided into four spatial zones that express completely different sets of OR genes and that appear to project axons to different zones in the olfactory bulb. Sensory neurons expressing the same OR are randomly distributed throughout one epithelial zone. Remarkably, this random distribution is transformed into an orderly pattern at the first brain relay center, the olfactory bulb. A highly organized spatial map is formed, wherein the axons of sensory neurons with the same OR terminate in one to three invariant glomeruli at two stereotyped sites, one of each side of the olfactory bulb. The result is that inputs from different ORs are segregated in different glomeruli. In the olfactory cortex, there is another, but very different, stereotyped map of signals derived from different ORs (Zou et al., 2001).

The molecular architecture of the olfactory system raises a number of intriguing developmental questions. One is how the olfactory sensory neuron selects a single OR gene to express from an array of 1000 candidate genes. It would appear that each neuron picks one OR allele from a zonal set of several hundred genes. OR genes expressed in the same zone are not clustered at one chromosomal site, arguing against a locus-dependent gene choice involving a single zonal enhancer (Sullivan et al., 1996), but the locations of putative zonal enhancers and the mechanisms underlying OR gene choice are not yet known.

Another puzzle is how neurons expressing the same OR, which are scattered in one epithelial zone, come to synapse in precisely the same few glomeruli in the olfactory bulb. When the coding region of one OR is replaced by another, the axons target to a novel glomerulus, indicating a critical role for the OR itself in targeting (Mombaerts et al., 1996; Wang et al., 1998). Given that different epithelial zones project axons to different zones in the olfactory bulb, it would seem that the axon first selects the appropriate bulb zone and then finds its target glomerulus. The locations of the novel glomeruli formed in the OR swap experiments are consistent with this idea. However, in two previous studies, neurons expressing large OR transgenes, either a 300 kb *M12* transgene (Ebrahimi et al., 2000) or a 460 kb *MOR28* transgene (Serizawa et al., 2000), targeted novel glomeruli, raising the possibility that elements, or even other genes, distant from an OR gene play a role in correct targeting in the olfactory bulb.

To explore the mechanisms underlying OR gene choice and axon targeting, Vassalli et al. (2002) examined the expression patterns and glomerular targeting of neurons that express OR minigenes of different sizes and composition. Glomerular convergence by neurons expressing the endogenous and transgenic alleles of a particular OR is elegantly demonstrated by tagging the two alleles with different histological markers and demonstrating that the cognate glomeruli receive fibers corresponding to both alleles. Vassalli et al. show that, for two OR genes, *MOR23* and *M71*, an ~9 kb minigene can recapitulate all features of the endogenous gene. The minigenes do not misexpress in multiple zones of the olfactory epithelium as does a previously described 6.7 kb *M4* transgene (Qasba and Reed, 1998), and the neurons expressing these minigenes do not project to ectopic glomeruli as do the previously reported 300 kb and 460 kb transgenes (Ebrahimi et al., 2000; Serizawa et al., 2000).

The authors then perform a deletion analysis of the 9 kb *MOR23* minigene, which consists of two noncoding 5′ exons, the *MOR23* open reading frame fused to an internal ribosome entry site and the histological reporter *taulacZ*, and a 3′ untranslated region. They find that the more proximal and larger of the two 5′ introns is dispensable for autonomous function. By contrast, the removal of both 5′ introns leads to a loss of zonal restriction in the olfactory epithelium, which is accompanied by innervation of ectopic glomeruli in addition to the cognate glomeruli. Importantly, the truncated transgenes that exhibit expression in multiple epithelial zones and project to ectopic glomeruli continue to obey the apparent global spatial correspondence between the zones of the olfactory epithelium and the olfactory bulb: expression in more ventrally situated epithelial zones leads to projection to more ventrally located glomeruli in the bulb (Mori et al., 2000).

Further loss of 1.7 kb 3′ of the coding region does not exacerbate the defects observed with the deletion of both 5′ introns. However, the compounded loss of 395 bp 5′ of the predicted transcriptional start site leads to a minigene that shows no expression in the olfactory epithelium. This 395 bp upstream region contains sequence motifs, such as the O/E and homeodomain bindings sites, which have been previously identified to be important for OR gene expression (Wang et al., 1997).

Together, these findings indicate that appropriate zonal expression of an OR gene does not necessarily

require a control element that is distant from the gene, though such an element could be positioned at different locations in or around different OR genes. They further imply that, consistent with previous observations (Mombaerts et al., 1996; Wang et al., 1998), targeting of axons to an appropriate zone in the olfactory bulb is dependent on the epithelial zone in which the neuron is located rather than the OR gene it expresses.

Vassalli et al. also report one curious finding. They find that a small fraction of neurons that express an endogenous OR allele innervate ectopic glomeruli that are formed by neurons in a different zone expressing the corresponding truncated allele. The authors propose that this "mistargeting" might result from homotypic interactions among the axons of neurons expressing the two alleles and thereby indicates an important involvement of such interactions in axonal targeting. Another possibility, however, is that neurons that express the endogenous allele but target ectopic glomeruli correspond to the small fraction of neurons that express a particular OR gene outside of its normal zone (Ressler et al., 1993). Quantitative analysis of the number of misrouted axons in the ectopic glomeruli and of the expression pattern of the endogenous *MOR23* allele in olfactory epithelia of the transgenic mice might help clarify which of the two models is more plausible.

Now that Vassalli et al. have shown that it is possible to construct a small transgene that can faithfully behave as an autonomous OR gene, the analysis of other such OR minigenes with subtler mutations, such as point mutations in putative transcription factor binding sites, can be attempted. The study of such mutated transgenes is likely to contribute to our understanding of the mechanisms underlying OR gene choice and shed further light on the determinants that govern glomerular convergence.

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Phasic Transmitter Release from Tonic Neurons

Graded and prolonged presynaptic depolarizations trigger the tonic release of neurotransmitters from sensory neurons. In this issue of *Neuron*, Simmons (2002a) reports that postsynaptic responses of locust interneuron synpapses are determined by the rate rather than the amplitude of presynaptic depolarization, suggesting a mechanism for increasing the signaling capabilities of this synapse with respect to visual processing.

Synapses which respond to graded membrane depolarization (as opposed to trains of all or nothing action potentials) are common components of the initial circuitry of sensory systems in both vertebrates and invertebrates, and synaptic depression has been shown to be an important mechanism for shaping signals in various sensory systems. However, while much is known about the properties of synaptic depression at synapses that respond to trains of impulses, relatively less is known about depression at synapses that respond to graded potentials. In this issue of *Neuron*, Simmons (2002a) uses the large locust sensory interneuron (L1-3) as a model system for examining depression at a synapse transmitting graded potentials.

Insects contain two distinct types of eyes: the compound eye and a small simple eye, the ocellus, both of which respond to light and visual input. The simple neural circuit of the non-image-forming ocellus includes the light-sensing photoreceptors, which connect to the L1-3 second-order interneurons (see Figure). The L1-3 interneurons in turn make both excitatory and inhibitory output synapses, which can easily be anatomically distinguished. L1-3 inhibitory synapses are reciprocal synapses between L1-3 and are located in the terminal arbors of L1-3, while L1-3 excitatory synapses are made onto third-order neurons such as DN (see Figure). In the ocellar circuit, a single presynaptic neuron, the L1-3, can thus excite one group of neurons and inhibit a separate set. Since the excitatory and inhibitory synapses made by L1-3 are located at anatomically distinct sites, it is possible to isolate each synapse for pre- and postsynaptic intracellular recordings.

In previous work, the author had shown that the inhibitory postsynaptic potentials (IPSPs) at L1-3 synapses decay rapidly. No matter what the length of the presynaptic depolarization is, the IPSPs were found to be transient. It was suggested that this transience might allow these synapses to be more sensitive to the rate of presynaptic depolarization than to the amplitude of the signal (Simmons, 2002b). In this report, the author sets out to test this directly via the technically demanding